NERL/MCEARD Publications

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Jan 1, 2000 - Dec 31, 2000

Presented Published

ABSTRCT/ORAL

Lindquist, H.D.A., Ware, M.W., Stetler, R.E., and Schaefer, III, F.W. Testing methods for detection of cryptosporidium spp. in water samples. Presented at: 3rd Seminar on Food and Waterborne Parasitic Zoonoses in the 21th Century, Bangkok, Thailand, December 6-8, 2000. — 12/7/2000

Contact: H. d. alan Lindquist

Abstract: A large waterborne outbreak of cryptosporidiosis in Milwaukee, Wisconsin, U.S.A. in 1993 prompted a search for ways to prevent large scale waterborne outbreaks of protozoan parasitoses. Two principle strategies have emerged: risk assessment leading to appropriate treatment and pathogen monitoring. Methods for detecting C. parvum play an integral role in both strategies.One criticism of current methods for detecting protozoa in water is that they produce results that are highly variable. It is difficult to determine if the methods themselves, or the procedures for testing them, are the source of the variability. If testing procedures are responsible for high variability, then the way in which methods are tested may not provide a fair comparison.To overcome this problem, the U.S. EPA has developed a set of criteria to aid in the evaluation of detection methods for parasites in water. Application of these criteria has elucidated some of the most important causes of testing variability. As a consequence, the Agency has developed approaches to overcome these pitfalls. The results from comparing several of these methods indicate that a monitoring strategy is not optimal at this time.

Lindquist, H.D.A. Current methods for detection of Cryptosporidium spp.. Presented at: Science and Mission Club, Cincinnati, OH, November 8, 2000. — 11/8/2000

Contact: H. d. alan Lindquist

Abstract: Current methods for detecting protozoa in water produce results that are highly variable. It is difficult to determine if the methods themselves, or the procedures for testing these methods, are the source of the variability. If testing procedures are responsible for high variability, then the way in which methods are tested may not provide a fair comparison. To overcome this problem, a set of criteria has been developd to aid in the evaluation of detection methods for parasites in water. Application of these criteria has elucidated some of the most important causes of testing variability. Approaches have been developed to overcome these pitfalls. Comparisons of several methods for detecting Cryptosporidium spp. in the environment, using more precise techniques, indicate differences in the efficiency of these methods.

Thompson, S., Jackson, J.L., Suva-Castillo, M., Yanko, W.A., and Williams, Jr., F.P. Detection of infectious adenovirus in tertiary treated and UV disinfected wastewater during a UV disinfection pilot study. Presented at: WEFTEC 2000, 73rd Annual Conference & Exposition, Anaheim, CA, October 14-18, 2000. — 10/15/2000

Contact: Frederick P. Williams

Abstract: An infectious enteric adenovirus was isolated from urban wastewater receiving tertiary treatment and ultraviolet (UV) disinfection. A pilot study was undertaken to investigate the efficacy of UV disinfection (low pressure, high intensity radiation) of total and fecal coliform bacteria, naturally occurring male-specific coliphage, seeded male-specific coliphage (MS20, naturally occurring enteirc viruses, and seeded poliovirus type 1 ((Lsc) in tertiary treated wastewater. The enteric viruses were concentrated from ca. 400 L of UV treated (20-70 mW-sec/cm2 doses) effluent using traditional adsorption elution methods and detected via a tissue culture cytopathic effects (CPE) assay. Sample concentrates were applied to monolayers of the CaCo-2 cell line and observed for a minimum of 14 days for CPE, followed by a minimum of 2 succeeding passages. Analysis of the UV treated samples for natural enteric viruses revealed the presence of a fastidious CPE-producing virus, later identified as an adenovirus by electron microscopic analysis. The adenovirus was isolated from a sample receiving a UV dose (ca. 60 mW-s/cm2) which reduced total and fecal coliform levels by ca. 5 Log10 units. Pilot unit data further showed that the same dose was capable of removing ca. 4 Log10 units of seeded MS2 coliphage. These results suggest that while UV disinfection may be effective at reducing levels of indicator bacteria, natural and seeded bacteriophage, and even seeded poliovirus, it may not provide equivalent reductions of some naturally occurring infectious enteric viruses such as adenovirus. Further research is need to determine appropriate doses for UV disinfection of enteric viruses in wastewater.

Jan 1, 2000 - Dec 31, 2000

Presented *Published*

Fout, G.S. Quality assurance for methods to detect human enteric viruses in drinking water. Presented at: Symposium on Viruses in Drinking Water, Seoul, Korea, October 6-14, 2000. 10/14/2000

Contact: G. shay Fout

Abstract: Surface or groundwaters impacted by untreated or inadequately treated domestic wastes may contain human pathogenic viruses that cause hepatitis, gastroenteritis, meningitis, encephalitis, myocarditis, diabetes, conjunctivitis and temporary or permanent paralysis. These viruses can and do cause outbreaks of waterborne disease. Of the 647 waterborne outbreaks that occurred in the U.S. between 1971 and 1996, 370 or 57 percent, involving 51,233 cases of illness, were caused by viruses or assumed to be caused by viruses. However, the disease burden to a community is generaly considered to be much higher than the number from reported outbreaks. Better estimates of the disease burden are needed in order to adequately protect the public. The use of occurrence studies is a common way to provide a better estimate. The most widely used method to perform occurrence studies on human enteric viruses in surface or drinking waters is the standard total culturable virus assay. This method detects a number of virus types that are endemic in many communities. Molecular methods have also been developed to detect these and other virus types that cause waterborne disease. All standard and molecular assays can generate false positive and false negative results. Minimizing these false results requires a good laboratory quality assurance program. The minimum quality assurance procedures that should be used with virus monitoring methods will be described.

Schaefer, F.W. A historical perspective of detection methods for giardia cysts and cryptosporidium oocysts in water. Presented at: USGS Workshop "Building Capabilities for Monitoring & Assessment of Public Health Microbiology", Columbus, OH, March 13-14, 2000. 3/14/2000

Contact: Frank W. Schaefer

Abstract: In the mid-20th century Giardia was classified as a non-pathogenic commensal organism and Cryptosporidium was not recognized yet. However as early as 1946 a waterborne outbreak of giardiasis was suspected. From 1965 to 1979 it became clear that Giardia lamblia was indeed a human pathoge and was responsible for waterborne gastroenteritis. Unlike bacteria, there are no simple culture methods for enteric protozoan parasites, so detection and identification were limited to microscopic observations. Because the densities of cysts and oocysts in water often can be less than one per liter, volumes between 10 and 1,000 liters must be examined. The initial sampling approach of the Centers for Disease Control and Prevention was to use a sand filter on a flatbed truck, while that of the U.S.EPA was to use a fiber wound cartridge filter which became the standard. Buoyant density flotation protocols employing zinc sulfate, sucrose, Percoll, and Percoll-sucrose have been used to purify and further concentrate the cysts and oocysts. Percoll-sucrose eventually became the standard flotation medium.

Fout, G.S. Occurrence of enteric viruses in surface waters. Presented at: USGS Workshop "Building Capabilities for Monitoring & Assessment of Public Health Microbiology, Columbus, OH, March 14-16, 2000. 3/14/2000

Contact: G. shay Fout

Abstract: Human enteric viruses cause a number of diseases when individuals are exposed to contaminated drinking & recreational waters. Vaccination against poliovirus has virtually eliminated poliomyelitis from the planet. Other members of enterovirus group cause numerous diseases. Hepatitis A and E have caused large waterborne hepatitis outbreaks. 2nd leading cause of illness in U.S. is acute nonbacterial gastroenteritis, which results from infection of suseptible individuals with members of caliciviridae, astroviridae, reviridae & adenoviridae families. First step in establishing risk of waterborne disease from these viruses is to determine levels of occurrence in contaminated waters. This requires methods to recover, identify and measure concentration of viruses in affected waters. Rapid polymerase chain reaction (PCR) methods have been developed to measure virus levels in environmental water. This study was performed to determine whether a multiplex PCR method developed to characterize the occurrence of enteroviruses, reoviruses, rotaviruses, hepatitis A and Norwalk virus in groundwater could be used iwth surface waters. The method was tested using surface waters from 5 USGS NAWQA Program sites selected to provide good geographic coverage of the U.S. and to span a range of hydroclimatic and land-use settings. All sites were found to be positive for two or more of the virus groups during the course of the study. The PCR findings from the NAWQA sites will be used to illustrate these problems and the proper interpretation of PCR-positive results.

Presented *Published*

Grimm, A.C., and Fout, G.S. Development of a molecular method to identify hepatitis E virus. Presented at: American Society for Virology, Ft. Collins, CO, July 8-12, 2000. 7/12/2000

Contact: Ann Grimm

Abstract: Hepatitis E virus (HEV) is a waterborne emerging pathogen that causes significant illness in the developing world. Thus far, an HEV outbreak has not been reported in the U.S., although a swine variant of the virus is common in Midwestern hogs. Because viruses isolated from two U.S. cases of human hepatitis E were very similar to the swine strain, it may be that HEV is a zoonotic virus. It will therefore be important to determine to what extent this virus may be present in potable water supplies. We have developed a reverse transcription-polymerase chain reaction method that should be able to identify all of the known HEV strains. When evaluated under laboratory conditions, this assay has detected low levels of all HEV strains

Lindquist, H.D.A. Surveying the risks from emerging diseases. Presented at: Dayton Chapter of the American Society of Civil Engineers Environmental Group, Dayton, OH, November 13, 2000. 11/13/2000

Contact: H. d. alan Lindquist

Abstract: Despite advances in sanitation and public health, new waterborne diseases have continued to cause outbreaks in humans. The reason why these organisms can cause disease outbreaks, is that their biology allows them to circumvent the safeguards put in place to prevent transmission of other diseases. Most of these agents of disease already exist in the environment, but become important causes of disease only when conditions change to favor their transmission. A number of emerging diseases will be discussed.

Schwegel, C.A., Gallagher, P.A., Wei, X., and Creed, J.T. Speciation and preservation of inorganic arsenic in drinking water supplies with IC-ICP-MS. Presented at: 2000 Winter Conference on Plasma Spectrochemistry, Ft. Lauderdale, FL, January 10-15, 2000. 1/11/2000

Contact: Carol A. Schwegel

Abstract: The speciation of inorganic arsenic in drinking water supplies is an essential part of devising an appropriate treatment process. Arsenate, because of its anion characteristics at drinking water pHs, is effectively removed by anion exchange treatment while arsenite remains in the treated drinking water. One of the issues in this analysis pertains to the preservation of the native As(III)/As(V) distribution during the transport of the sample to the laboratory. Some of the potential problems are due to changes in dissolved oxygen and waters containing elevated concentrations of iron. This poster will evaluate preservation techniques designed to curcumvent the redox changes which occur during shipment.

Rosenblum, L. Comparison of five extraction methods on incurred and fortified pesticides in composite diets: blender, soxhlet, ASE, microwave and SFE. Presented at: Florida Pesticide Residue Workshop, Tampa, FL, July 16-19, 2000. 7/19/2000

Contact: Laura G. Rosenblum

Abstract:

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Presented *Published*

Hedrick, E.J., Minges, H., Lee, T.G., and Biswas, P. Impinger solutions for the efficient capture of gaseous mercury species using direct injection nebulization inductively coupled plasma mass spectrometry (DIN-ICP/MS) analysis. Presented at: Air and Waste Management Association Meeting, Salt Lake City, UT, June 18-21, 2000. 6/18/2000

Contact: Elizabeth J. Hedrick

Abstract: Currently there are no EPA reference sampling mehtods that have been promulgated for measuring Hg from coal combustion sources. EPA Method 29 is most commonly applied. The ASTM Ontario Hydro Draft Method for measuring oxidized, elemental, particulate-bound and total Hg is now undergoing evaluation and review. Both these methods employ acidic permanganate impingers to capture elemental Hg and use cold vapor atomic absorption spectrometry for analysis (CVAA). Elemental Hg is oxidized in the permanganate solution and reduced back to elemental Hg for CVAA analysis. Accurate estimates of total Hg emissions depends entirely on the efficiency of the impingers to oxidize, capture and stabilize Hg in solution. In this work inductively coupled plasma mass spectrometry (ICP/MS) using direct injection nebulization (DIN) is used to evaluate novel impinger solution compositions that more efficiently capture elemental and oxidized Hg. The work presented will describe analytical capabilities, experimental set-up and results from impinger solution experiments. Results from a thorough investigation of the application of DIN-ICP/MS for routinely measuring total Hg in a variety of solution matirces will be presented as well as a comparison to CVAA. Various impinger solution compositions to selectively capture Hg species were efaluated by generating elemental Hg vapor and HgCl2, entraining the gases in a stream of air and measuring how much Hg could be captured in the impinger solutions. DIN-ICP/MS allowed for the evaluation of solutions not amenable to CVAA. Evidence will be presented that indicates the acidified permanganate solution employed in EPA Method 29 and the Ontario Hydro Method may not capture Hg very efficiently. Implications for accurately measuring Hg in coal combustion emissions will be presented.

Julien, E.A., Berry, Jr., M.R., Tomerlin, J.R., Sert, M.Y., Tucker, K.D., and Waylett, D.K. Recent enhancements to the dietary exposure potential model. Presented at: ISEA Meeting, Monterey, CA, October 24-27, 2000. 10/25/2000

Contact: Maurice R. Berry

Abstract: Presentation describes recent enhancements & new applications of the Dietary Exposure Potential Model (DEPM), a model developed to assist in design & interpretation of dietary exposure measurements. Model is an interactive system that provides dietary exposure estimates using data from established food consumption and residue databases. Dietary exposure to a chemical may be estimated for U.S. population and for 20 sub-populations defined by various demographic characteristics. DEPM has been updated to include additional consumption data, and residue data from a variety of chemical classes. Recent enhancements add considerable flexibility to the program. The user may combine residue data from multiple databases. Also user may modify data reported in DEPMs resident databases and may incorporate data for chemicals & foods that are not included in any of databases. Analysis of contribution of tap water to total exposure is also possible. In addition user may incorporate food consumption data from daily intake diary studies such as from files created using electronic food coding software. Although not intended for risk assessment DEPM provides preliminary estimates of dietary exposure and can be used to compare exposure estimates across ages, sex, reigons, etc.; provide preliminary estimates of relative importance of diet to total exposure; and indicate food items or consumption patterns primarily responsible for exposure to specific residues.

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Presented *Published*

Rohrer, C., Hieber, T., Melnyk, L.J., and Berry, Jr., M.R. Pesticide transfer efficiency from household surfaces to foods. Presented at: ISEA Annual Conference, Monterey, CA, October 24-27, 2000. 10/24/2000

Contact: Lisa J. Melnyk

Abstract: Application of pesticides around homes presents a potential for exposure to young children. Contaminated surfaces can be contacted by children's hands or foods which could allow transfer of pesticides. The exposures caused by these contacts are uncertain because the amount of pesticide transferred is unknown. This study determined the percent transfer efficiency from household surfaces to specific food items using an isopropanol wipe and a C-18 filter contained on a dermal press sampler to quantify pesticide surface residues. Commercial aqueous formulations of the target pesticides were applied by pipette to hardwood flooring, ceramic tile and carpet. Foods were contacted with each surface for 1, 10 and 60 minutes with and without addtional force applied. Duplicate surfaces were wiped and pressed to determine the levels of pesticides available on the surfaces for transfer. Additional surfaces were contacted with bread, chicken nuggets, fries and bananas for 10 min without added force. The highest transfer efficiencies for all pesticides occurred for apples contacting hardwood flooring. Individual pesticide transfer efficiency varied significantly for each surface. For example, malathion, chlorpyrifos and isofenphos transfer efficiencies from hardwood to apple were greater than 60% and diazinon and permethrins were <15% for a contact time of 10 minutes without contact force. Minimal transfer of pesticides from carpet were measured from either wipes or foods. Generaly, increased contact time and applied contact force to the foods increased transfers of pesticides for any surface. For example, cis and transfer permethrin transfer efficiencies increased <15% to around 50% when contact force was applied.

Morgan, J.N., and Kauffman, P. Comparison of GC-FPD and GC/AED for determination of organophosphate pesticides in composite diet samples. Presented at: Eastern Analytical Symposium, Atlantic City, NJ, October 29 - November 3, 2000. 10/29/2000

Contact: Jeffrey N. Morgan

Abstract: In order to assess an individual's total exposure to contaminants in the environment, it is essential that the contribution of dietary exposure be quantified. As a result, USEPA's National Exposure Research Laboratory has initiated a program to develop methods to measure chemical pollutants in dietary samples collected from individuals. Methods developed previously utilizing GC/MS-SIM for pesticides in composite diets are unsuitable for many organophosphorus pesticides. Consequently the present study was undertaken to evaluate the suitability of GC/FPD and GC/AED for determination of organophosphorus pesticides in composite diets. Composite diet samples containing three levels of fat (1, 5 and 10%) and fortified with 37 organophosphorus pesticides were extracted with a methylene chloride-acetone solvent mixture using accelerated solvent extraction and cleaned up using diatomaceous earth and C18 reversed phase chromatography. Pesticides were quantitated by capillary GC using a DB-17 column with both flame photometric detection (phosphorous mode) and atomic emission detection. Recovery of fortified pesticides, precision (% relative standard deviation) and method detection limits were determined using both GC/FPD and GC/AED. Recoveries ranged from 30-93% for 37 pesticides studied. Recoveries from high fat composite diets were greater than 70% for 30 of 37 pesticides. RSDs were generally below 15%. Recoveries were poor for naled, methamidophos, acephate, chlorethoxyfos, dichlorvos, and disulfoton. In general GC/FPD was more snesitive than GC/AED for most analytes tested. Specific results will be presented along with a discussion of advantages and disadvantages of each technique.

Creed, J.T., Gallagher, P.A., Wei, X., Shoemaker, J.A., and Schwegel, C.A. Speciation of arsenic in exposure assessment matrices. Presented at: 23rd Annual Conference on Analysis of Pollutants in the Environment, Pittsburgh, PA, May 14-16, 2000. 5/14/2000

Contact: John T. Creed

Abstract: The speciaton of arsenic in water, food and urine are analytical capabilities which are an essential part in arsenic risk assessment. The cancer risk associated with arsenic has been the driving force in generating the analytical research in each of these matrices. This presentation will provide the highlights of our in-house research effort in each of these matrices over the last year.

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Presented *Published*

Creed, J.T., Gallagher, P.A., Wei, X., Schwegel, C.A., Lorenzana, R.M., and Chamberlain, I. Accelerated solvent extraction of arsenicals from seafood matrices with ion chromatography and ICP-MS detection. Presented at: 4th International Conference on Arsenic Exposure and Health Effects, San Diego, CA, June 18-22, 2000. 6/19/2000

Contact: John T. Creed

Abstract: The two major sources of arsenic exposure are water and diet. Dietary exposure is considerably more difficult to assess because of the diversity of arsenicals present in dietary matrices coupled with species dependent toxicity of arsenic. Dietary arsenic assessments are further complicated by an incomplete understanding of all analytical factors which assure species specific integrity from extraction to detection and variation in extraction efficiencies. Near quantitative extractions may be achieved on a Standard Reference Material while the extraction of similar matrices produces extraction efficiencies of <25%. In this case, 75% of arsenicals remains unextracted. This type of variance in extraction efficiency within the same type of matrix is one reason extraction of arsenicals from dietary matrices is still an active area of research. One approach to extraction of arsenicals from seafood sources is accelerated solvent extraction (ASE). This approach allows for optimization of solvent, pressure, temperature and static time. The ASE will be evaluated in terms of its ability to quantitatively extract arsenicals from seafoods using conventional solvents while maintaining species integrity. The ASE is a semi-automated extraction device in which the sample is loaded into a stainless steel cell after it has been dispersed in a dispersion media. The appropriate choice of cell components and dispersion media will be presented with an emhasis on QC samples. This evaluation wll include chromatographic concerns resulting from addition of arsenosugars to potential list of extractable arsenicals and use of ICP-MS as a detector.

Freeman, N.C.G., Pellizzari, E.D., Melnyk, L.J., and Berry, Jr., M.R. Relationships of metals in floor dust and on the hands of toddlers. Presented at: ISEA Annual Conference, Monterey, CA, October 24-27, 2000. 10/27/2000

Contact: Lisa J. Melnyk

Abstract: Toddlers are characterized by their frequent hand-to-mouth activity and exploratory behavior. This puts them at risk for exposure to environmental contaminants. Wipe samples are traditionally used to collect dust samples in homes. The assumption is that dust samples are indicative of what the child contacts and that loadings on children's hands should be similar to those found in the child's environment. As part of the Children's Dietary Lead Study hand wipes were obtrained from 45 toddlers; floor wipes were collected at the same time, with the same sampling medium. Analysis of wipe samples was conducted by ICP-MS for aluminum, arsenic, barium, cadmium, chromium, copper, lead, manganese, nickel, vanadium and zinc. No detectable chromium was found in any wipe sample. Aluminum and lead were found in all floor and hand wipe samples. Lead loadings (ng/cm2) on floor and hand wipes were correlated and were not significantly different in amount. In contrast, aluminum loadings on hands were not correlated with loadings from floor wipes and were significantly greater in amount. Median loadings were 413 and 71 ng/cm2 respectively. For many of the metals the propoRtion of samples with detectable levels was similar for hand wipes and floor wipes. However, arsenic and vanadium were found in more than 80% of hand wipe samples, yet less than 50% of floor wipe samples. For most of the metals, hand loadings were significantly greater than floor loadings. Two factors that may contribute to these differences are the multiple sources of exposure that the child experiences and differential particle size distributions that adhere to hands and are found on floors. The results suggest that for many metals hand loadings may exceed loadings found in environmental samples, and therefore are better indicators of exposure.

Cantu, R., Evans, O.M., Kawahara, F.K., and Dufour, A.P. Enhanced retention and sensitivity in the analysis of cyanuric acid in water using porous graphitic carbon and UV detection in high pressure liquid chromatography. Presented at: American Chemical Society 220th National Meeting, Washington, DC, August 20-24, 2000. 8/20/2000

Contact: Otis M. Evans

Abstract: Cyanuric acid (CA) has found application as a chlorine stabilizer in pool waters. The National Swimming Pool Foundation recommends CA levels between 30-50 ppm and a chlorine residual of 1.0-3.0 ppm. These chlorine levels are needed to destroy harmful pathogenic organisms. Developing a rugged method to monitor CA in water is crucial in maintaining adequate chlorine levels that do not pose undue hazards to human health. Existing methodology employing HPLC has proven ineffective because of lack of CA retention imposed by use of silica based columns. A rugged method has been developed using porous graphitic carbon (PGC) to analyze real world water samples. The analysis employed 955 phosphate:5% methanol at pH 7.4 with UV detection at 213 nm. The CA retention factor (K') using PGC was 8 while for Cl8, C8, C6H5, NH2, and CN silica columns it was unsuitable (<0.1). Raising the pH to 9.2 resulted in practical retention (k'=4) and gave 20% more sensaitivity due to optimum UV detection.

Jan 1, 2000 - Dec 31, 2000

Presented *Published*

Hu, Y., Akland, G.G., Pellizzari, E.D., Clayton, C.A., Melnyk, L.J., and Berry, Jr., M.R. Dietary exposures of young children, part 3: modelling. Presented at: ISEA Annual Meeting, Monterey, CA, October 24-27, 2000. 10/27/2000

Contact: Lisa J. Melnyk

Abstract: A deterministic model was used to model dietary exposure of young children. Parameters included pesticide residue on food before handling, surface pesticide loading, transfer efficiencies and children's activity patterns. Three components of dietary pesticide exposure were included: the amount of pesticide in foods before handling, amount transferred from contaminated surface to foods, and amount transferred from contaminated hands to foods. Monte Carlo sensitivity analysis was performed to identify the most sensitive parameters and to guide laboratory studies and a field study of 3 children from homes that recently applied diazinon. Sensitivity analysis indicated the most sensitive parameter was surface pesticide loading, followed by surface-to-hand transfer and pesticide residue on food consumed. Transfer efficiencies estimated in laboratory experiments were in range of 0-80% for various tested surfaces and foods. These estimates were applied in the model to provide a preliminary assessment of dietary pesticide exposure. The modeling results indicated that pesticide transfer to food caused by contact with surfaces and handling could increase the pesticide intake significantly. Depending on a child's behavior pattern while eatring, food type and surface type, a child's handling of food can contribute >40% of excess pesticide intake. These results were evaluated in a field study in which environmental and biologcial measuement samples were collected. The validity of the model prediction was examined n two ways: by comparison of pesticide in leftover handled foods as predicted by the model and actual field measurements; and by comparison of overnight urinary diazinon metabolite after exposed and unexposed days. also included analysis of the model uncertainty using Monte Carlo method, and limitations associated with the pilot field study for model validation.

Raymer, J.H., Pellizzari, E.D., Akland, G.G., Weinberg, H., and Shoemaker, J.A. The uptake of water disinfection by-products into foods during home processing. Presented at: ISEA Annual Meeting, Monterey, CA, October 24-27, 2000. 10/24/2000

Contact: Jody A. Shoemaker

Abstract: A variety of organic compounds in tap water are produced as a result of disinfection process. Use of chlorine-containing chemicals for disinfection produces many disinfection by-products (DBPs) including trihalomethanes, haloacetonitriles and haloacetic acid. Ozonation with secondary disinfectants such as hypochlorite yields many of same compounds although at lower concentrations, and a variety of aldehydes, ketones and bromate (if the source water contains bromide). Human ingestion exposures to DBPs occur not only as a result of drinking disinfected water, but also by using tap water to prepare foods/beverages in the home. Work underway at RTI is designed to improve our understanding of contamination of foods as a result of cooking food in water containing DBPs. We evaluated the stability of haloacetic acids (HAAs) and haloacetonitriles in water during boiling and measured the uptake of HAAs into foods cooked by different methods. Foods tested: spaghetti, pinto beans, instant oatmeal, chicken, carrots and green beans. Reagent water was spiked with bromochloroacetic, bromodichloroacetic, chlorodibromoacetic, dibromoacetic, dichloroacetic, monobromoacetic, monochloroacetic and tribromoacetic, and trichloroaceti acids. After 60 min of boiling only chloroacetic acid and dichloroacetic acid were well-recovered. The cooking experiments showed a total of 2-9.6% of the HAAs from cooking water were absorbed into pasta (unrinsed) and significant amounts of chloroacetic, bromoacetic, dichloroacetic, bromochloroacetic and dibromoacetic acids were lost upon rinsing with reagent water. However, when rinsing water contained HAAs a net increase in HAA concentrations in pasta was measured. The concentrations of HAAs not stable to cooking also increased following rinsing.

Jan 1, 2000 - Dec 31, 2000

Presented Published

Melnyk, L.J., Berry, Jr., M.R., Akland, G.G., Hu, Y., and Pellizzari, E.D. Dietary Exposures of young children, part 1: model development and study design. Presented at: ISEA Annual Conference, Monterey, CA, October 24-27, 2000. 10/24/2000

Contact: Lisa J. Melnyk

Abstract: Young children contact surfaces (hands, floors, etc.) that may be contaminated with pesticides. Thus, dietary exposures of young children are difficult to measure, but are needed to support the aggregate exposure assessments. Evaluation of dietary field protocols and a total dietary intake model was the goal of this study. Diazinon with a half life of 8 hrs was chosen as the primary pesticide in order to detect a difference in exposure as indicated by the amount of metabolite in urine samples on a daily basis. Other samples collected to determine the model parameters included a 24-hr duplicate-diet, leftover touched and untouched foods, food-surface samples, food-press sampler samples, an activity diary, video tape, hand prints and wipes, surface wipes and press, indoor/outdoor air, entrance soil, and morning urine. One to three year old children were recruited based on diazinon use in their home and a surface wipe screening of diazinon at levels greater than 5 ng/cm2. Sampling was scheduled for six consecutive days following the day of applicabion. The child performed routine activities on exposed days. Controlled activities for eating and handling foods by the child were monitored on alternate unexposed days to determine if differences in exposure were detectable using food, environmental, and morning urine samples. Video tapes were analyzed to obtain contact frequencies and durations. The various samples were successfully collected and dietary exposures calculated from the collected samples. The estimated total dietary exposure calculated from the model will determine if the model adequately predicts dietary exposures of chidlren to pesticides when incorporating their activities.

Hu, Y., Pellizzari, E.D., Raymer, J.H., Akland, G.G., Beach, J.B., Keever, J.T., Barr, D, Needham, L., Schumacher, B.A., and Melnyk, L.J. Collecting urine samples from young children for pesticide studies. Presented at: ISEA Annual Conference, Monterey, CA, October 24-27, 2000. 10/26/2000

Contact: Lisa J. Melnyk

Abstract: To estimate pesticide exposure for young children wearing diapers, a method for collecting urine samples for analysis of pesticide metabolites is needed. To find a practical method, two possibilities were investigated: (1) analysis of expressed urine from cotton diaper inserts and (2) analysis of the whole void extracted from the diaper (or other urine collection materials). In the first study, the validity of using cotton gauze pads as diaper inserts to collect urine samples from young children was tested. Urine was spiked with a pesticide and four metabolites: 2,4-dichlorophenoxyacetic acid (which is mainly eliminated from urine unchanged), 3-phenoxybenzoic acid (a metabolite of synthetic pyrethroids), atrazine mercapturate (a metabolite of atrazine), malathion dicarboxylic acid (a metabolite of malathion) and 2-isopropyl-4-methyl-6-hydroxypyrimidine (a metabolite of diazinon). Aliquots of the spiked urine were added to the gauze pads and were expressed from the pads, using a syringe, immediately and after 1,2,4,8 and 24 hours to determine the impact of contact time on recovery. Expressed urine samples were then extracted and analyzed. The recoveries of target analytes from the expressed urine were within a range of 70-130%.In the second study, potential urine collection materials such as cloth diaper, sanitary napkins, gel sorbers and clay were spiked with a list of 12 potential urinary metabolites of organophosphate and carbamate pesticides. The urine was extracted and analyzed. The recoveries for each tested material were determined and will be presented.

Jan 1, 2000 - Dec 31, 2000

Presented Published

Akland, G.G., Pellizzari, E.D., Hu, Y., Whitaker, D., Melnyk, L.J., Berry, Jr., M.R., and Leckie, J. Dietary exposures of young children, part II: field study. Presented at: ISEA Annual Conference, Monterey, CA, October 24-27, 2000. 10/25/2000

Contact: Lisa J. Melnyk

Abstract: A small, pilot field study was conducted to determine the adequacy of protocols for dietary exposure measurements. Samples were collected to estimate the amount of pesticides transferred from contaminated surfaces or hands to foods of young children and to validate a dietary model which was developed to estimate children's dietary exposures.Three homes were selected for study. Each home had a young child (1-3 years old), and diazinon had recently been applied, which was confirmed by surface wipe samples. To meet the specific aims, sampling occurred over six consecutive days following the date of pesticide application. During the six day monitoring period, environmental samples (e.g., air, surface wipe and surface press), personal exposure samples (e.g. hand wipe), biological samples (e.g. morning urine samples), food samples (e.g. duplicate diet, leftover handled food), questionnaires and activity video were collected. On "exposed days" the child ate his or her meals without any constraints imposed by the experiment. On alternating days ("unexposed days"), the child's hands were washed thoroughly before eating, and then the child ate from a clean surfae (either a place mat or hihg chair which was provided by RTI). Sentinel foods, such as apples, bananas, bread, and hot dogs, were handled by the child on the exposed and non-exposed days for comparison. A continuous dust (particle) monitoring device with data logger was used to measure air particulate concentratoins throughout the monitoring period as an indicator of aerosol generation and/or resuspension related to on-going activities occurring within the home. Twenty four hour indoor and outdoor integrated air samples were collected each of the six consecutive days of monitoring. Samples for surface pesticide loading were collected at the beginning, middle and end of the monitoring period. Duplicate diet, leftover and handled food and morning urine samples were collected daily. Morning urine collections from diaper wearers were made using a newly developed method for this study.The pilot study demonstrated that the field protocols provide useful data for dietary model validation and for assessing the potential contribution of surface, hands, and food interactions on total dietary exposure.

Glassmeyer, S. The effects of disinfection on pharmaceuticals in drinking water supplies. Presented at: Society of Environmental Toxicology and Chemistry, Nashville, TN, November 11-16, 2000. 11/12/2000

Contact: Jody A. Shoemaker

Abstract: Pharmaceuticals are intended to be applied to or ingested by humans and animals, metabolized and excreted through urine or feces. However it has been estimated that 30-90% of administered active ingredients pass through humans and animals unchanged. While sewage treatment plants remove between 7-96% of 14 pharmaceuticals (mean 65+/-25) not all pharmaceutical-containing waters pass through sewage treatment plants before drinking water treatment (e.g. terrestrial/aquatic applicaitons of veterinary pharmaceuticals) so potential exists for pharmaceuticals to be in the source water of drinking water treatment plants. There are very few studies in the literature concerning effect of drinking water treatment on fate of pharmaceutical compounds, and those that are present were conducted in urban areas where sewage and drinking water treatment are presumably state-of-the-art. The purpose of this study was to examine a range of human and veterinary pharmaceuticals to estimate the amount of active ingredient that makes it through simple water treatment unchanged, as well as identify any disinfection byproducts/degradation nproducts that may form. Compounds studied cover a wide variety of drug classes including non-steroidal anti-inflammatory drugs (NSAIDs, acetaminophen, ibuprofen), antibiotics (amoxicilllin, vancomycin, tetracyccline), antihistamines (cimetidine, pseudoephedrine), hormones (levothyroxine, depo-provera), antiarrhythmic agents (digoxin), and antineoplastics (levamisole HCl), as well as other compounds that would have an anthropogenic origin (caffeine, nicotine, aspartame) and some known metabolites (paraxanthine, -(-cotinine)).

Jan 1, 2000 - Dec 31, 2000

Presented *Published*

Cantu, R., Evans, O.M., and Dufour, A.P. Rapid and simplified HPLC method with UV detection, pH control and selective dechlorinator for cyanuric acid analysis in water. Presented at: American Chemical Society Regional Meeting, Covington, KY, May 16-19, 2000. — 5/16/2000

Contact: Otis M. Evans

Abstract: Cyanuric acid (CA) and chloroisocyanurates are commonly used as standard ingredients in formulations for household bleaches, industrial cleansers, dishwasher compounds, general sanitizers, and chlorine stabilizers. They are very well known for preventing the photolytic decomposition of chlorine disinfectant. HPLC methods have been favored for CA analysis. However, striking variations in pH (1-10, most in the neutral range 6.7-7.8) and UV detection (196-225 nm) among these methods demand an operational method with pH control, practical sensitivity, and CA retention. This method is now fully developed as CA is measured adequately still using the HPLC-UV technique but with optimum parameters. UV spectra of CA show strong pH dependency with a strong 213 nm absorption at pHs>7. The HPLC setup combined a C18 separation column with 5% methanol/95% phosphate eluents over a wide array of pHs (2-10). Capacity factors were computed from 0.057 to 0.478. Practical sensitivity and intermediate retention were fond at pH 7.3. The complex equilibria of chlorinated isocyanurates in solution (up to 10 species) are eliminated to 2 species (CA and first ion) by dechlorination with ascorbic acid and watEr reaction resulting in chlorine liberation and CA product as confirmed by UV, HPLC-UV and LC-MS techniques. The proposed method exploits fundamental chemical equilibria, reactivity, and optical properties of CA to measure it in the practical 0.5-125 ug/ml range with an MDL of 0.05 ug/ml.

Rosenblum, L. Comparison of five extraction methods on incurred and fortified pesticides in composite diets: blender, soxhlet, ASE, microwave and SFE. Presented at: AOAC Central Regional Meeting, Columbus, OH, November 1-3, 2000. — 11/2/2000

Contact: Laura G. Rosenblum

Abstract: The USEPA National Exposure Research Laboratory studies dietary exposure to a diverse group of semi-volatile pesticides by analyzing 24 hour duplicate composite diets. The pesticides of interest include organochlorines, organophosphates, anilines, and triazines. Currently, there are no official methods for pesticide analysis of composite samples that include fatty and non-fatty foods. Composite diets consisting of multiple food items with field-incurred or laboratory-fortified pesticides were created to compare extraction efficiency by Soxhlet, blender, microwave, ASE, and SFE. Similar pesticide recoveries were obtained by all extraction methods, though the methods varied widely in instrument cost, extraction time, and solvent use. Co-extractants were removed using a novel clean-up method that included pre-separation in the large volume injector of a GC/MS. The extraction, clean-up, and determinative steps permit the analysis of non-polar and moderately polar pesticides in fatty samples with a method detection limit of about 1 ng/g for thermally stable semi-volatile pesticides and reduce solvent consumption.

Jan 1, 2000 - Dec 31, 2000

Presented *Published*

Gallagher, P.A., Schwegel-Brockhoff, C.A., Murray, S., Creed, J.T., Wei, X., McKiernan, J.W., and Caruso, J.A. Accelerated solvent extraction of arsenicals from environmental matrices with ion chromatography separation and ICP-MS detection. Presented at: 2000 Winter Conference on Plasma Spectrochemistry, Ft. Lauderdale, FL, January 10-15, 2000. 1/11/2000

Contact: John T. Creed

Abstract: The two major sources of arsenic exposure used in an arsenic risk assessment are water and diet. The extraction, separation and quantification of individual arsenic species from dietary sources is considered an area of uncertainty within the arsenic risk assessment. The uncertainty stems from the lack of species specific information on arsenic from a number of food groups. Arsenic's species dependent toxicity has generated the need for speciation based methodologies. A considerable amount of research in arsenic speciation has been focused on the separation and detection of arsenicals. Recently, the structural information has become increasingly necessary as the list of potential arsenicals continues to grow and chromatographic separation capabilities have somewhat plateaued. An additional area which seems to be lagging behind is the quantitative extraction of arsenicals from dietary samples and composite diet samples. The quantitative extraction is essential in assuring that the extraction procedure is removing all of the toxic species and not selectively removing only the non-toxic arsenicals. Using an extraction procedure which is nearly quantitative allows for a more definitive risk assessment. Seafood has been assessed to be one of the highest dietary sources of total arsenic. This aspect makes the investigation and identification of arsenicals in seafood and seaweed an exposure source issue for arsenic. One extraction technique which has been applied to the extraction of arsenicals from seafoods is Accelerated Solvent Extraction (ASE). ASE has recently been utilized as a semi-automated technique that allows for optimization via solvent temperature and pressure as well as solvent composition and static time. The development of an analytical procedure surrounding ASE requires the need for certain quality control (QC) samples to be analyzed. The Laboratory Fortified Blank (LFB) is one of these QC samples. The analysis of a LFB by the ASE requires placing a spike of the analytes in a blank ASE cell containing the appropriate support matrix. Poor percent recoveries of LFBs have led to a detailed investigation of the mechanics of the ASE extraction. This presentation will focus on a complete verification of this technique presented in terms of recoveries of LFBs and Laboratory Fortified Matrices (LFMs). This will include an evaluation of the frits, filters, dispersion media, extraction solvents and the appropriate blowdown technique.

Gallagher, P.A., Schwegel, C.A., Creed, J.T., heck, amy, and Wei, X. A comparison of extraction efficiencies in seafood matrices using a synthetic stomach and an accelerated solvent extraction approach with IC-ICP-MS detection. Presented at: European Winter Conference on Plasma Spectrochemistry, Lillehammer, Norway, February 4-8, 2001. 2/6/2000

Contact: John T. Creed

Abstract: Seafood is one of the largest sources of dietary arsenic exposure. Because most of the arsenic present is non-toxic (such as arsenobetaine [AsB]), the consumption of seafood is thought to result in a low risk or non-toxic exposure. This can be misleading for two reasons. First, while toxic arsenicals (such as monomethylarsonic acid [MMA], dimethylllarsinic acid [DMA], and inorganic arsenic) may be present in seafoods at fractional levels, the total arsenic concentration can exceed 50 ppm. At these high levels, the fractional component results in microgram doses of toxic arsenic species. Secondly, toxic arsenicals can represent 50% of the total arsenic present in certain kelp based seafoods. This exposure could easily exceed microgram quantities of toxic arsenic species but a typical ingestion of kelp is limited to gram quantities. In either case, these exposure to toxic arsenicals are comparable to those predicted from drinking water using an arsenic maximum contaminant level [MCL] of 5 ug/L and 2 L/day consumption rates. Therefore, seafood ingestion can be a signficant exposure route for toxic arsenicals and the estimation of this exposure improves the reliability of the overall arsenic risk assessment.

Jan 1, 2000 - Dec 31, 2000

Presented *Published*

Creed, J.T., Gallagher, P.A., Wei, X., and Schwegel, C.A. Extraction techniques for the removal of arsenicals from seafood exposure matrices with ICP-MS detection. Presented at: 4th International Symposium on Speciation of Elements in Biological, Environmental and Toxicological Sciences, Vancouver, Canada, June 25 - July 1, 2000. 6/26/2000

Contact: John T. Creed

Abstract: Most of the existing arsenic dietary databases were developed from the analysis of total arsenic in water and dietary samples. These databases have been used to estimate arsenic exposure and in turn human health risk. However, these dietary databases are becoming obsolete as the distribution of toxic and non-toxic arsenicals in various food groups becomes better understood through research in arsenic speciation. Seafoods have been a target food group because of the relatively high total arsenic concentration associated with seafoods indicate that the arsenicals present are non-toxic. This generality can be misleading in certain seafoods because the extraction efficiency of the arsenicals can be relatively low and/or the toxic species have been found to be a large percentage of the total arsenic extracted. In order to move from this generality toward a preliminary database established from speciation data, analytical methods with near quantitative extraction along with good species specific preservation are essential. Methods which document their performance via fortified matrices are required to begin to generate a preliminary database of known quality. Research to date has indicated that the analyses of seafood samples is strongly marix dependent. This results in speciation data in which the speciated fraction represents less than half of the available arsenic. Therefore, the arsenic risk information associated with this data would require a qualifier which indicates that over 50% of the arsenic is of unknown toxicity. In an attempt to move toward a more quantitative extraction, alternative weak or soft digestion and enzymatic approaches have been investigated. These results will be reported with a special emphasis on species specific integrity.

Creed, J.T., Gallagher, P.A., Schwegel, C.A., and Heck, Amy. Arsenic speciation: sampling preservation and analysis. Presented at: Water Quality Technology Conference, Salt Lake City, UT, November 5-9, 2000. 11/5/2000

Contact: John T. Creed

Abstract:

Wymer, L.J., and Dufour, A.P. A model for estimating the incidence of swimming-related gasrointestinal illness as a function of water quality indicators. Presented at: 4th International Conference on Environmetrics and Chemometrics, Las Vegas, NV, September 18-20, 2000. 9/18/2000

Contact: Larry J. Wymer

Abstract: Several studies have demonstrated association between gastrointestinal illness (GI) in swimmers and sewage pollution as measured by the density of indicator organisms, such as e. coli and enterococci, in recreational waters. These studies generally classify illnesses into two categories according to the subjectivity of the reported symptoms and utilize separate analyses on the incidence of total illness and the incidence of objective symptoms of gastroenteritis. In addition, non-swimmer illness rates are available from these studies as an indicator of the background illness rates, but are not always utilized in the model. Ordinal logistic regression using response levels corresponding to the severity of illness is shown herein to be a potentially useful technique for modeling such data when background rates are included. Data from two prospective epidemiological studies conducted by the EPA and evidencing relationships between the incidence of swimming-associated GI and enterococcus or e. coli density in marine and fresh water, respectively, are used as examples. Initially, analysis of these data consisted of linear regression of log10 enterococcus density on the difference in illness rates between swimmers and non-swimmers. Subsequent published analysis of the marine study utilized logistic regression, but did not take background illness rates into account. both analyses produced separate models for rates of "highly credible" and total GI symptoms. The present analysis indicates that including the background rates improves the fit and that a proportional odds assumption for the dose-response is justified for these data.

Jan 1, 2000 - Dec 31, 2000

Presented *Published*

Santo Domingo, J., Newby, T., and Harmon, S.M. Evaluation of the use of different antibiotics in the direct viable count method to detect fecal enterococci. Presented at: American Society of Microbiology, Los Angeles, CA, May 21-25, 2000. 5/23/2000

Contact: Jorge Santo-domingo

Abstract: The detection of fecal pollution is performed via culturing methods in spite of the fact that culturable counts can severely underestimate the densities of fecal microorganisms. One approach that has been used to enumerate bacteria is the direct viable count method (DVC). The objective of this study was to evaluate the use of nalidixic acid, priomidic acid, pipemidic acid, ciprofloxacin, and cephalexin in the DVC method to measure viability of fecal enterococci. The effect of the anitbiotics on five environmental isolates was examined using flow cytometry, scanning electron microsocpy, epifluorescence microscoy, and image analysis. Not all of the antibiotics tested induced an increase in the isolates cell size, even after eight hours of incubation with elevated antibiotic concentrations. Although differences in the general response to the antibiotic treatment were observed between starving and nonstarving cells, in general the combination of ciprofloxacin and cephalexin was more effective in promoting increase in cell size than the individual antibiotics. Additionally, cell biovolume was found to be a more sensitive indicator of viability for fecal enterococci than cell length and surface area.

Brenner, K.P., Siefring, S.D., and Brown, S.L. Comparison of mEnterococcus agar and the U.S. Environmental Protection Agency-recomended Enterococci methods, mE and mEI agar. Presented at: American Society of Microbiology, Los Angeles, CA, May 21-24, 2000. 5/24/2000

Contact: Kristen P. Brenner

Abstract: To maintain waters that are "fishable and swimmable", mandated by the Clean Water Act, the U.S. Environmental Protection Agency (EPA) is developing a list of approved methods for use in enumerating enterococci and E. coli in ambient waters. As part of this effort, we compared mEnterococcus agar (mENT), using fresh water samples and 43 different incubation schemes, with the EPA-recommended methods, mE and mEI agar, incubated at 41oC for 48 and 24 h, respectively. Although the mean recoveries of the 2 EPA methods were similar at their recommended incubation times, the recovery with mEI agar was greater than that of mE agar at 24 h and that of the verified mE recovery on Esculin-Iron agar. Both EPA methods recovered more enterococci than mENT incubated at 35oC and ast 35oC and 44.5oC. The mean 41oC mENT recoveries at 24 and 48 h were similar to those of mE agar, and the concentration at 48 h was similar to that of mEI agar. The 41oC mENT method shows promise for use in water quality monitoring programs along with the current recommended enterococci methods.

McDaniels, A.E., Stelma, Jr., G.N., and Haugland, R.A. Quantitative measurement of Helicobacter pylori by the TaqMan fluorogenic probe system. Presented at: American Society of Microbiology, Los Angeles, CA, May 21-24, 2000. 5/22/2000

Contact: Audrey E. Mcdaniels

Abstract: Culturing of H. pylori from environmental sources continues to be an obstacle in detecting and enumerating this organism. Successful methods of isolation and growth from water samples have not yet been developed. In this study a method involving real tme PCR product detection with a fluorogenic hybridization probe (TaqMan) was evaluated for use in quantifying H. pylori cells. Selection of primer and probe sequences for the ureA gene was performed based on comparative sequence analyses of 16 strains of H. pylori and other Helicobacter species. Samples of serially diluted H. pylori cells spanning a 6-log concentration range were subjected to DNA extraction and TaqMan analysis. Estimated cell quantities in the extracted samples ranged from 20 to 2 x 10-6 based on direct microscopic counts following staining with DAPI. Results from 5 replicate experiments showed a good correlation between TaqMan assay results (expressed as cycle threshold values) and the logarithms of expected cell numbers based on direct counts over the entire cell quantity range tested. Similar results were seen for the two Helicobacter pylori strains. It was concluded that the TaqMan quantitative PCR method has the potential to provide accurate quantification of H. pylori cells in environmental samples.

Jan 1, 2000 - Dec 31, 2000

Presented *Published*

Haugland, R.A., Vesper, S.J., and Wymer, L.J. A rapid method for the extraction of fungal DNA from environmental samples: evaluation in the quantitative analysis of Memnoniella echinata conidia using real time detection of PCR products. Presented at: Mycologcal Society of American Annual Meeting, Burlington, VT, July 29 - August 3, 2000. 7/30/2000

Contact: Richard A. Haugland

Abstract: New technologies are creating the potential for using nucleic acid sequence detection to perform routine microbiological analyses of environmental samples. Our laboratory has recently reported on the development of a method for the quantitative detection of Stachybotrys chartarum conidia in air and indoor dust samples using a primer and probe set that is specific for ITS region rDNA sequences from this organism and the TaqMan fluorigenic probe assay. This system allows analyses of up to 96 samples to be performed in two hours. The high sample throughput permitted by this system has lead us to search for more streamlined methods for preparing suitable DNA extracts for analysis. In the current study we describe a modification of our previously reported method for the extraction of DNA from fungal conidia that reduces the overall sample processing time by approximately one-half. The new method continues to use bead milling to disrupt the fungal cells but substitutes a commercially available manifold-based DNA purification system for the previously used centrifugation-based system. We also describe a new TaqMan primer and probe set for the specific detection of the toxigenic fungal species, Memnoniella echinata. DNA extractions by the new method were found to support similar levels of accuracy and precision in the quantification of M. echinata conidia in both liquid and dust matrices to those previously reported for S. chartarum using the longer extraction method. Ninety-five percent occurrence ranges of these quantitative estimates were within 50 to 200% of actual cell numbers in the liquid samples and 20 to 65% in the dust samples over a range from 25 to 1000 cells per sample.

Brenner, K.P. Methods for determining recreational water quality. Presented at: USGS Workshop "Building Capabilities for Monitoring & Assessment in Public Health Microbiology", Columbus, OH, March 14-16, 2000. 3/14/2000

Contact: Kristen P. Brenner

Abstract: The goal of the clean water act of 1972 was to restore and maintain physical, chemical & biological quality of waters in the U.S. Although great progress has been made in cleaning up lakes, rivers and coastal waters many still do not meet water quality standards. Most beaches have been closed for at least one day because of high bacterial concentrations or other sources of contamination. In 1986 EPA recommended the use of two new membrane filter methods, mE agar and mTEC agar for monitoring recreational water for enterococci and e.coli, because the previous indicator orgaanisms, total and fecal coliforms, were not specific for fecal contamination. The recommendation was based on epidemiological studies that showed illness rates were directly related to enterococci and e.coli concentrations in the water, but not fecal coliform concentrations. Enterococci levels were correlated with illness rates in both fresh and marine recreational water while e.coli concentrations were correlated in fresh water only. Since then, two improved methods have been developed: mEI agar method for enterococci and modified mTEC agar method for e.coli. These methods allow faster and easier enumeration of target microorganisms. The mEI method is able to recover the same number of enterococci in 24 hours that mE method recovered in 48 hours, and modified mTEC method eliminated the filter transfer step to a second medium. However, results from both of these methods are not available until 24 hours after samples are collected, and other enterococci methods cited in the literature may take up to 72 hours. This means detection of unsafe levels of indicator organisms in recreational water occurs after exposure to the swimmers, bathers and other users. Therefore, rapid methods with results obtained the same day the sample is taken, preferably within hours, are needed to quickly assess the condition of recreational water so the public can be warned of risk of possible exposure to pathogens.

Presented ***Published***

Brinkman, N., Haugland, R.A., Santo Domingo, J., and Vesper, S.J. Detection and identification of pathogenic candida species in water using flow cytometry coupled with taqman pcr. Presented at: American Society for Microbiology, Orlando, FL, May 20-24, 2000. — 5/20/2000

Contact: Nicole Brinkman

Abstract: As the incidence of human fungal infection increases, the ability to detect and identify pathogenic fungi in potential environmental reservoirs becomes increasingly important for disease control. PCR based assays are widely used for diagnostic purposes, but may be inadequate for analyses of environmental samples due to the presence of inhibitors. Another method, flow cytometry, allows samples to be rapidly screened for organisms that exhibit target antibodies. These organisms can be recovered using the single cell sorting capability of the flow cytometer. The limitation of the flow cytometry method is that different related and even unrelated species may share the target characteristics used for sorting and thus cannot be distinguished. In this study we tested the use of flow cytometry for isolation of pathogenic Candida species from water samples coupled with TaqMan PCR for identification of the recovered organisms. Using flow cytometry, it was demonstrated that a fluorescently labeled Candida species, including C. albicans, C. tropicalis, C. parasilosis, C. viswanathii, C. sojae, C. dubliniensis, C. maltosa, C. haemulonii, and C. lusitaniae exhibit sufficiently higher fluorescence levels than unrelated yeasts to allow their selective recovery. Different TaqMan PCR assays for each of these target species were shown to be specific and were indicated to be sensitive enough to detect as few as 1 - 20 cells per reaction, depending on the target species. The combination of the two methods described here provides a useful approach for identifying pathogenic Candida species in environmental samples.

BOOK

Hurst, C.J., and Lindquist, H.D.A. "Defining the ecology of viruses." In: Viral Ecology, Chapter 1 C.J. Hurst (Ed.), San Diego, CA: Academic Press 2000. EPA/600/A-00/030. — 5/1/2000

Contact: H. d. alan Lindquist

Abstract:

Covert, T.C. "Salmonella. In American Water Works Association Manual of Water Supply Practices, Waterborne Pathogens, AWWA M48." In: Waterborne Pathogens, AWWA Manual M48, Chapter 15 Denver, Colorado: AWWA 2000, 107-110. EPA/600/A-00/029. — 1/1/2000

Contact: Terry C. Covert

Abstract:

JOURNAL

Eberhard, M.L., Ortega, Y.R., Hanes, D.E., Nace, E.K., Do, R.Q., Robl, M.G., Won, K.Y., Gavidia, C., Sass, N.L., Mansfield, K., Gozalo, A., Griffiths, J., Gilman, R., Sterling, C.R., and Arrowood, M.J. Attempts to establish experimental cyclospora cayetanensis infection in laboratory animals. June 2000. Journal of Parasitology 86 (3):577-582 (2000). — 6/1/2000

Contact: Frank W. Schaefer

Abstract: Attempts were made to develop an animal model for Cyclospora cayetanensis to identify a practical laboratory host for studying human cyclosporiasis. Oocysts collected from stool of infected humans in the United States, Haiti, Guatemala, Peru and Nepal were held in potassium dichromate solution to allow development of sporozoites. The following animal types were inoculated: 9 strains of mice, including adult and neonatal immunocompetent and immune-deficient inbred and outbred strains, rats, sandrats, chickens, ducks, rabbits, gerbils, hamsters, ferrets, pigs, dogs, owl monkeys, rhesus monkeys and cynomolgus monkeys. Most animals were inoculated by gavage, although some of the primates were fed oocysts on food items. The animals were examined for signs of infection, particularly diarrhea, and stool samples were examined for 4-6 wk after inoculation. None of the animals developed patent infections or signs of infection. We conclude that none of the animals tested are susceptible to infection with C. cayetanensis.

Hester, J.D., Lindquist, H.D.A., Bobst, A.M., and Schaefer, F.W. Fluorescent in situ detection of encephalitozoon hellem spores with a 6-carboxyfluorescein-labeled RNA-targeted oligonucleotide probe. May-June. Journal of Eukaryotic Microbiology (Society of Protozoologists) 47 (3):299-308 (2000). EPA/600/J-00/132. 5/1/2000

Contact: H. d. alan Lindquist

Abstract: A fluorescent in situ hybridization assay has been developed for the detection of the human-pathogenic microsporidian, Encephalitozoon hellem, in water samples using epifluorescence microscopy. The assay employs a 19-nucleotide species-specific 6-carboxyfluorescein-labeled oligonucleotide probe, HEL878F, designed to be complementary to the nucleic acid sequence 878-896, a highly variable segment of the 16S ribosomal RNA of E. hellem spores. The specificity of this probe for its ribosomal RNA target site was confirmed using RNA degradation, ribosomal RNA target site competition, and nucleotide base mismatch control probe assays. Furthermore, the specificity of the HEL878F oligonucleotide probe for E. hellem spores was established when it was evaluated on spores from all three species of the genus Encephalitozoon that had been seeded in reagent water and environmental water concentrates. The specificity of the HEL878F oligonucleotide probe was further corroborated when tested on algae, bacteria, and protozoa commonly found in environmental water. The study demonstrates the applicability of a fluorescent in situ hydridization assay using a species-specific fluorescent-labeled oligonucleotide probe for the detection of E. hellem spores in water samples.

Melnyk, L.J., Berry, Jr., M.R., Sheldon, L.S., Freeman, N., Pellizzari, E.D., and Kinman, R.N. Dietary exposure of children living in lead-laden environments. Journal of Exposure Analysis and Environmental Epidemiology 10 (No. 6, pt. 2):723-731 (2000). EPA/600/J-01/010. 11/1/2000

Contact: Lisa J. Melnyk

Abstract: Children are the most susceptible population to lead exposure because of three interacting factors: they have more opportunity for contact with lead sources due to their activities; lead absorption occurs more readily in a child as compared to an adult; and the child's development is more vulnerable to lead than adults. Low levels of lead in the blood have been shown to cause adverse health effects; the level of concern for children in currently 10 ug/dL (CDC, 1991). The contribution of dietary exposure of lead to increased blood lead levels (PbB) is not well characterized. This study was conducted with experimental techniques to obtain estimated dietary lead intakes of children 2 - 3 years of age who live in homes contaminated with environmental lead. Research objectives were to estimate potential lead intakes for children consuming food in contaminated enivornments recognizing unstructured eating patterns and to investigate potential correlations between daily dietary exposure to lead and measured blood levels. Dietary exposure was evaluated by collecting food samples that were representative of the foods the young children ate in their homes. A 24-hr duplicate of all foods plus sentinel foods, i.e., individual food items used to represent foods contaminated during handling, were collected from 48 children. Ten of the participants were revisited to obtain information on the variation in daily dietary intakes. Drinking water was evaluated both as part of the segregated beverage sample composite and by itself. Additional information collected included lead concentrations from hand wipes, floor wipes, and venous blood; and questionnaire responses from the caregiver on activities potentially related to exposure.

Cantu, R., Evans, O.M., Shoemaker, J.A., and Dufour, A.P. Rapid and simplified HPLC method with UV detection, pH control and reductive ascorbic acid for cyanuric acid in water. Analytical Chemistry (American Chemical Society) 72 (23):5820-5828 (2000). EPA/600/J-01/014. 12/1/2000

Contact: Ricardo Cantu

Abstract: Every year over 250 million pounds of cyanuric acid (CA) and chloroisocyanurates are produced industrially. These compounds are standard ingredients in formulations for household bleaches, industrial cleansers, dishwasher compounds, general sanitizers, and chlorine stabilizers. The method developed for CA using high performance liquid chromatography (HPLC) with UV detection simplifies and optimizes certain parameters of previous methodologies by effective control of pH of the eluent (95 % phosphate buffer : 5 % methanol, v/v) to the narrow pH range of 7.2-7.4. UV detection was set at the optimum 213 nm wavelength intercept where the cyanuric ion absorbs strongly. Analysis at the lower pH range of 6.8-7.1 proved inadequate due to CA keto-enol tautomerism, while at pHs < 6.8 there were substantial losses in analytical sensitivity. In contrast, pHs > 7.4 proved more sensitive but their use was rejected because of CA elution at the chromatographic void volume and due to chemical interferences. The complex equilibria of chlorinated isocyanurates and associated species was suppressed using reductive ascorbic acid to restrict the products to CA. UV, HPLC-UV, and electrospray ionization/mass spectrometry were combined to monitor reactive chlorinated isocyanurates and to support the use of reductive ascorbic acid. The resulting method measures CA in the 0.5-125 mg/L linear concentration range with a method detection limit of 0.05 mg/L in water.

Presented *Published*

Raymer, J.H., Pellizzari, E.D., Childs, B., Briggs, K., and Shoemaker, J.A. Analytical methods for water disinfection by-products in foods and beverages. Journal of Exposure Analysis and Environmental Epidemiology (New York, NY: Nature Publishing Group) 10 (No. 6, pt. 2):808-815 (2000). EPA/600/J-01/011. 11/17/2000

Contact: Jody A. Shoemaker

Abstract: The determination of exposure to drinking water disinfection byproducts (DBPs) requires an understanding of how drinking water comes in contact with humans through multiple pathways. In order to facilitate the investigation of human exposure to DBPs via foods and beverages, analytical method development efforts were initiated for haloacetonitriles, haloketones and chloropicrin and the haloacetic acids in these matrices. The recoveries of target analytes were investigated from composite foods and beverages. Individual foods and beverages used to investigate the general applicability of developed methods were selected for testing based on their water content and frequency of consumption. The haloacetonitriles, the haloketones and chloral hydrate were generally well-recovered except for bormochloroacetonitrile and dibromacetonitrile from foods spiked after homogenization and following extraction with methyl-t-butyl ether, the addition of acetone was found to be necessary to improve recoveries from beverages. The process of homogenization resulted in decreased recoveries for the more volatile analytes despite the presence of dry ice. The haloaceitc acids were generally well-recovered except for trichloroacetic acid and tribromoacetic acid from foods but low recoveries and emulsion formation were experienced with some beverages. With both groups of analytes, certain matrices were more problematic (as measured by volatility losses, emulsion formation) than others with regard to processing andl anayte recovery.

Wei, X., Schwegel, C.A., and Creed, J.T. Application of sample pre-oxidation of arsenite in human urine prior to speciaton via on-line photo-oxidation with membrane hydride genration and ICP-MS detection. Analyst (Cambridge, United Kingdom: The Royal Society of Chemistry) 125 (6):1215-1220 (2000). EPA/600/J-01/016. 5/30/2000

Contact: John T. Creed

Abstract: A pre-oxidation procedure which converts arsenite (AS(III)) to arsenate (As(VVV)) was investigated in urinary arsenic speciation prior to on-line photo-oxidation hydride-generation with ICP-MS detection. This sample pre-oxidation method eliminates As(III) and As(V) preservation concerns, simplified & facilitates chromatographic separations. Four oxidants were investigated. Chlorine and MnO2 selectively convErted As(III) to As(V) in pure water samples, but conversion was inefficient in complex urine matrix. Oxidation of As(III) by H202 was least affected by urine matrix, but removal of excess H2O2 at pH 10 proved difficult. The most appropriate oxidant for selective conversion of As(III) to As(V) with minimal interference from urine matrix is I3 at pH 7. Unlike H202, excess oxidant can be easily removed by addition of S2032. The I3/S2032 treatment on a fortified sample of reconstituted NIST SRM 2670 freeze dried urine indicated arsenobetaine (AsB), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and As(V) were not chemically degraded with percent recoveries ranging from 95-102% for all arsenicals. Sample clean-up involved pH adjustment prior to C18 filtration in order ot achieve efficient (As(III) conversion and quantitative recoveries of AsB and DMA. The concentrations determined in NIST SRM 2670 freeze dried urine were AsB 17.2+/-0.5, DMA 55.6+/-4.1, MMA 10.3+/-0.3 with a combined total of 8.31+/-5.0 (ug/L+/-2o).

Hu, Y., Barr, D, Akland, G.G., Melnyk, L.J., Needham, L., Pellizzari, E.D., Raymer, J.H., and Roberds, J.M. Collecting urine samples from young children using cotton gauze for pesticide studies. Journal of Exposure Analysis and Environmental Epidemiology (New York, NY: Nature Publishing Group) 10 (No. 6, pt. 2):703-709 (2000). EPA/600/J-01/013. 11/1/2000

Contact: Lisa J. Melnyk

Abstract: To estimate pesticide exposure, urine samples are often needed to analyze pesticide metabolites. However, this is difficult for children wearing diapers because simple and feasible techniques suitable for field collection are not available. The objectives of this study were to test the validity of using cotton gauze pad as a medium for collecting urine samples from young children and to examine the stability of the recoveries for creatinine and pesticide metabolites over 24 hours. Urine spiked with a pesticide and four metabolites, 2,4-dichlorophenoxyacetic acid (which is mainly eliminated from urine unchanged), 3-phenoxybenzoic acid (metabolite for synthetic pyrethroids), atrazine mercapturate (metabolite for atrazine), malathion dicarboxylic acid (metabolite for malathion) and 2-isopropyl-4-methyl-6-hydroxypyrimidine (metabolite for diazinon) was added to the gauze pads and kept in jars at 37oC in a water bath. Urine was expressed from the gauze pads immediately and after 1, 2, 4, 8 and 24 hours, then analyzed. The recoveries, calculated as the percentage of concentration in expressed urine divided by that of the control urine sample, were within a range of 70-130%. The metabolite and creatinine concentrations did not change with time in either expressed urine samples or controls. The results suggest that cotton gauze pad is a promising candidate for collecitng urine samples from young children wearing diapers for studies in which these five urinary pesticide metabolites are to be analyzed.

Akland, G.G., Pellizzari, E.D., Hu, Y., Roberds, M., Rohrer, C., Leckie, J., and Berry, Jr., M.R. Factors influencing total dietary exposure of young children. Journal of Exposure Analysis and Environmental Epidemiology (New York, NY: Nature Publishing Group) 10 (No. 6, pt. 2):710-722 (2000). EPA/600/J-01/012. 11/1/2000

Contact: Maurice R. Berry

Abstract: A deterministic model was developed to identify critical input parameters to assess dietary intake of young children. The model was used as a framework for understanding important factors in data collection and analysis. Factors incorporated included transfer efficiencies of pesticide from surfaces to food, from surfaces to hands to food, and more accurate microactivity data related to contact frequency for three variables of interest--hands, surfaces and food. Results from range finding measurements of transfer efficiencies using aqueous pesticide solution of a mixture of malathion, diazinon and chlorpyrifos sprayed on surfaces indicate a higher pesticide transfer occurred from hard surfaces to food (hardwood, plastic) with low transfer from soft surfaces (carpet, cloth). Six children under 4 years old were videotaped to obtain realistic contact frequency and times for interaction of hands, surfaces and foods during meals and snacks in their homes or day care centers. Range of eating events varied from 2-55 minutes (avg 20 minutes). Avg. number of contacts between food/hands was 19 for each eating event (range 10-40). Contacts between surface/hand were about the same. Contacts between food/surfaces ranged from 0-32, but only 5 or less were associated with surfaces other than eating utensil. Children's microactivity data collected during eating events with laboratory results from transfer studies were provided as input into a Monte Carlo simulation of dietary ingestion model. Simulation results indicate that children's handling of food could contribute 20-80% of total dietary intake of pesticides. Dietary exposure due to residues in food before handling accounted for 16 and 47% respectively of total mean intake from simulations for a child's consumption of an apple or banana. Results indicate transfer efficiencies for foods on various surfaces as well as hand contacts with food/surfaces are important as determinants of dietary exposure.

Gallagher, P.A., Wei, X., Shoemaker, J.A., Schwegel, C.A., and Creed, J.T. Detection of arsenosugars from kelp extracts via IC-ESI-MSS/MS and IC membrane hydride generation ICP-MS. Journal of Analytical Atomic Spectrometry (Cambridge, United Kingdom: The Royal Society of Chemistry) 14 (12):1829-1834 (2000). EPA/600/J-01/015. 1/1/2000

Contact: John T. Creed

Abstract:

Jan 1, 2000 - Dec 31, 2000

Presented Published

Argueta, C., Yoder, S., Holtzman, A.E., Aronson, T.W., Glover, N., Berlin, O.G.W., Stelma, Jr., G.N., Froman, S., and Tomasek, P. Isolation and identification of nontuberculous mycobacteria from foods as possible exposure sources. Journal of Food Protection 63 (7):930-933 (2000). 7/1/2000

Contact: Gerard N. Stelma

Abstract: A variety of foods collected from local supermarkets and produce stands were examined as possible sources of nontuberculous mycobacterial exposure. Food samples were combined with sterile ultrapure water and manually shaken. To remove large particles, the suspensions were filtered through a sterile strainer, centrifuged, and the supernatants were discarded. The food pellets were stored at -75oC. The pellets were treated with either oxalic acid or sodium hydroxide-sodium citrate solutions to reduce contamination by nonmycobacterial organisms. Decontaminated pellets were cultured on both Middlebrook 7H10C agar and Middlebrook 7H10C agar with supplemental malachite green. Plates sere observed for growth at 2 and 8 weeks. Isolates demonstrating acid-fastness were identified to species using polymerase chain reaction and restriction enzyme analyzsis. Nontuberculous mycobacteria (NTM) were recovered from 2 of 121 foods. Six different species of NTM were isolated, the most predominant being Mycobacterium avium.

Vesper, S.J., Dearborn, D.G., Elidemir, O., and Haugland, R.A. Quantification of siderophore and hemolysin from stachybotrys chartarum strains, including a strain isolated from the lung of a child with pulmonary hemorrhage and hemosiderosis. Applied and Environmental Microbiology 66 (6):2678-2681 (2000). EPA/600/J-00/149. 6/1/2000

Contact: Stephen J. Vesper

Abstract: A strain of Stachybotrys chartarum was recently isolated from the lung of a pulmonary hemorrhage and hemosiderosis (PH) patient in Texas (designated the Houston strain). This is the first time that S. chartarum has been isolated from the lung of a PH patient. In this study, the Houston strain and 10 strains of S. chartarum isolated from case (n-5) or control (n-5) homes in Cleveland were analyzed for hemolytic activity, siderophore production, and relatedness as measured by random amplified polymorphic DNA analysis.

Santo Domingo, J., Harmon, S.M., and Bennett, J.W. Survival of salmonella species in river water. Current Microbiology (New York, NY: Springer-Verlag) 40:409-417 (2000). EPA/600/J-00/144. 6/1/2000

Contact: Jorge Santo-domingo

Abstract: The survival of four Salmonella strains in river water microcosms was monitored by culturing techniques, direct counts, whole-cell hybridization, scanning electron microscopy, and resuscitation techniques via the direct viable count method and flow cytometry. Plate counts of bacteria resuspended in filtered and untreated river water decreased several orders of magnitude within the first week of incubation, while they did not decrease as rapidly in autoclaved water. In situ hybridization studies suggested a rapid decrease in ribosomal content, as determined by the drastic decrease in the number of detectable cells after 72h. In contrast, direct counts remained relatively constant during 45 days in all microcosoms. Although the culturable counts of two bacterial strains in filtered water after 31 days represented approximately 0.001% of the total counts, direct viable counts and resuscitation studies with a dilution series suggested that the number of viable bacteria was at least four orders of magnitude higher. Additionally, notable changes in forward scatter and in nucleic acid content were observed only after 4 h of nutrient amendments by flow ccytometry. However, cells from the resuscitation experiments did not grow on solid media unless cell-free supernatant from viable cultures was added during the resuscitation period. The results in this study suggest the presence of a not immediately culturable status in Salmonella.

Vesper, S.J., Dearborn, Yike, I., Allan, T., Sobolewski, J., Hinkley, S.F., Jarvis, B.B., and Haugland, R.A. Evaluation of stachybotrys chartarum in pulmonary hemorrhage case house before, during and after remediation. 2000. Journal of Urban Health 77:68-85 (2000). 5/1/2000

Contact: Stephen J. Vesper

Abstract:

Jan 1, 2000 - Dec 31, 2000

Presented Published

PUB REPORT

Behymer, T.D., and Munch, D.J. Methods for the determination of organic and inorganic compounds in drinking water, volume 1. 2000. EPA/815/R-00/014. — 8/31/2000

Contact: Thomas D. Behymer

Abstract:

Munch, J.W. Method 528. Determination of phenols in drinking water by solid phase extraction and capillary column gas chromatography/mass spectrometry (GC/MS). 2000. EPA/815/R-00/014. — 8/1/2000

Contact: Jean W. Munch

Abstract:

Brenner, K.P. Membrane filter method for the simultaneous detection of total coliforms and escherichia coli in drinking water. 2000. EPA/600/R-00/013, [NET]. — 3/1/2000

Contact: Kristen P. Brenner

Abstract:

SYMPOS/CONF

Zhuang, Y., Biswas, P, Quintan, M.E., Lee, T.-G., and Hedrick. Kinetic study of adsorption and transformation of mercury on fly ash particles in an entrained flow reactor. Presented at: Air & Waste Management Association National Conference, Salt Lake City, UT, June 18-21, 2000. — 6/20/2000

Contact: Elizabeth J. Hedrick

Abstract: Experimental studies were performed to investigate the interactions of elemental mercury vapor with entrained fly ash particles from coal combustion in a flow reactor. The rate of transformation of elemental mercury on fly ash particles was evauated over the temperature range from 303K to 780K. The reaction was approximately first order (0.91 for total fly ash, and 0.98 for submicrometer fly ash), and the reaction rate increased with increasing temperature. The results indicate that fly ash particles have sufficient minerals to promote the heterogeneous transformation of mercury. Experiments were then conducted with pure titania, silica, and iron oxide particles. Single component particle experiments (titania, silica and iron oxide) were performed to establish their contributions to the transformation of elemental mercury. Iron oxide caused the conversion of elemental mercury more efficiently than that of fly ash particles, while titania and silica did not result in oxidation or adsorption of the elemental mercury.

Hedrick, E.J., Minges, H., Lee, T.-G., and Biswas, P. Evaluation of iodine based impinger solutions for the efficient capture of Hg using direct injection nebulization inductively coupled plasma mass spectrometry (DIN-ICP/MS) analysis. Presented at: Air & Waste Management Association National Conference, Salt Lake City, UT, June 18-22, 2000. — 6/19/2000

Contact: Elizabeth J. Hedrick

Abstract: Currently there are no EPA reference sampling methods that have been promulgated for measuring stack emissions of Hg from coal combustion sources, however, EPA Method 29 is most commonly applied. The draft ASTM Ontario Hydro Method for measuring oxidized, elemental, particulate-bound and total Hg and the draft Tris-Buffer method have undergone evaluation and are favored over EPA Method 29 for accurate speciation of elemental and oxidized Hg. All of these methods employ acidic permanganate impingers to capture elemental Hg and use cold vapor atomic absorption spectrometry for Hg analysis (CVAA). Elemental Hg is oxidized in the permanganate solution and reduced back to elemental Hg for CVAA analysis. In this work inductively coupled plasma mass spectrometry (ICP/MS) using direct injection nebulization (DIN) was used to evaluate novel impinger solution compositions capable of capturing elemental Hg. The impinger solution compositions were selected to be amenable to ICP/MS analysis and results were compared to the Ontario Hydro Method. DIN-ICP/MS proved to be very specific for Hg, as sensitive as CVAA and has the potential benefit of allowing direct multi-element analysis of peroxide and iodine based impinger solutions. Hydriodic acid (HI) and acidcified potassium iodide were most efficident at capturing elemental Hg and were amenable to ICP/MS analysis.

Jan 1, 2000 - Dec 31, 2000

Presented Published

Rodgers, M.R., Shadix, L., and Feige, M.A. Studies on the use of ampicillin-dextrin agar asas aeromonas recovery medium. Presented at: Water Technology Conference, Salt Lake City, UT, November 5-9, 2000. 11/6/2000

Contact: Mark R. Rodgers

Abstract: The Contaminant Candidate List (CCL) includes the unregulated chemical and microbiological contaminants the EPA has identified as possibly posing a significant public risk to consumers if present in drinking water (1). There are three bacterial species listed in the CCL (Aeromonas hydrophila, Mycobacterium avium and Helicobacter pylori) and more information is needed on the occurrence of all of these species in drinking water in the U.S. Aeromonas are Gram negative, facultatively anaerobic, rod shaped bacteria in the family Aeromonadaceae, that occur ubiquitously in surface waters. These bacteria are considered to be human pathogens, although there is a disagreement as to the specific spectrum of disease caused by Aeromonas. While there are documented cases of Aeromonas septicemia, it is less clear whether Aeromonas are causative agents of gastroenteritis (2). It is generally agreed upon that of the 15 different recognized groups/species in this genus, only three, A. hydrophila, A. caviae and A. veronii biotype sobria, can be considered to be major human pathogens (2). Many methods have been published for the recovery of Aeromonas bacteria from environmental waters. Havelaar et.al. developed such a method using ampicillin dextrin agar (ADA), in 1987 and several reports subsequently showed it to be one of the best media for recovery of Aeromonas bacteria (3,4,5). ADA employs both antibiotic, ampicillin, and a detergent, deoxycholate, as selective agents. Most Aeromonas are intrinsically resistant to ampicillin and deoxycholate. This medium also contains dextrin as a fermentable sugar and bromthymol blue as a pH indicator. Since all aeromonads ferment dextrin, producing acid by-products, they appear on ADA as yellow colonies. Other non-dextrin fermenting bacteria appear green or colorless on ADA. ADA is compatible with a membrane filtration procedure, allowing the bacteria from large volumes of water to be concentrated and grown on membrane filters.

Jan 1, 2000 - Dec 31, 2000

	Presented	Published

JOURNAL

Lindquist, H.D.A., Bennett, J.W., Ware, M.W., Stetler, R.E., Gauci, M., and Schaefer, III, F.W. Testing methods for detection of Cryptosporidium spp. in water samples. 3rd Seminar on Food and Waterborne Parasitic Zoonoses in the 21st Century, Bangkok, Thailand, December 6-8, 2000. Southeast Asian Journal of Tropical Medicine and Public Health (Bangkok, Thailand: SEAMEO Regional Tropical Medicine &Public Health Network) 32 (2):190-194 (2001). — 12/6/2000 — 2/1/2001

Contact: H. d. alan Lindquist

Abstract: A large waterborne outbreak of cryptosporidiosis in Milwaukee, Wisconsin, U.S.A. in 1993 prompted a search for ways to prevent large-scale waterborne outbreaks of protozoan parasitoses. Methods for detecting Cryptosporidium parvum play an integral role in strategies that lead to appropriate treatment of surface water, but are criticize because they produce results that are highly variable.The U.S. Environmental Protection Agency developed a set of criteria to evaluate detection methods for protozoan parasites in water. As a consequence, the Agency has had to develop approaches to reducing uncertainty of evaluations. The variability and accuracy of various methods of producing small numbers of Cryptosporidium spp. oocysts were tested. The least variable and most accurate method was used to spike seven surface water, and one tap water sample to compare 4 detection methods that had been reported in the literature. The least variable and most accurate method for spiking specified numbers of oocysts into samples was found to be flow cytometry. The most effective of the methods tested for detection in both environmental and reagent water was solid phase cytometry.